THE EFFECT OF DIMETHYLSULFOXIDE ON THE INFECTIVITY OF THE T1-COLIPHAGE

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Master of Science in Biology

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by

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RECORD OF THESIS

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Title: THE EFFECT OF DIMETHYLSULFOXIDE ON THE INFECTIVITY OF THE T1-COLIPHAGE

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ABSTRACT OF THESIS

THE EFFECT OF DIMETHYLSULFOXIDE ON THE INFECTIVITY OF THE T1-COLIPHAGE

In this study it was observed that when the T1-coliphage:Escherichia coli B system contained 10% dimethylsulfoxide (DMSO) there were significantly more plaques than in control plates. At other DMSO concentrations there was no significant increase in the number of plaques. Sucrose and glycerol were substituted for DMSO, and the sucrose-treated system produced fewer plaques than the control. On the other hand, the glycerol did increase the number of plaques to a limited extent.

The observed effect of producing more plaques by the addition of 10% DMSO was observed on a 16 hr inoculum, but no significant difference was noted with a 7 hr inoculum.

Through biochemical analyses it was determined that a DMSO extract of E. coli B cells contained some protein and perhaps some lipid. This action of dissolving cell wall constituents may render the E. coli B cell more susceptable to viral attack.

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July 22, 1971
Date

Accepted by the faculty of the School of Sciences and Mathematics, Morehead State University, in partial fulfillment of the requirements for the Masters of Science degree.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Until the present time, studies concerning the T1-coliphage have been very limited as compared to the extensive studies conducted on other T-coliphages. However, this study is intended to add a significant piece to the entire puzzle of the T1-coliphage.

The T1-coliphages were mixed with Escherichia coli B cells and 10% dimethylsulfoxide (DMSO) and plated with controls. The plaque titration technique was used to determine whether or not DMSO would cause an increase in the infectivity of the virus. It was determined after counting 100 experimental and 100 control plates, that 10% DMSO increased the infectivity of the T1-coliphage by 10.8%.

It was also found that 10% DMSO dissolves some protein and possibly some lipid from the E. coli B cell wall. This segment of the study attests to the vast solvent properties of DMSO. These solvent properties will be applied often in many fields, and now cryobiologists are using DMSO to protect living tissues from freezing damage. It is expected in the years to come that many important uses for dimethylsulfoxide will be discovered.

LITERATURE REVIEW

EARLY STUDIES

The study of bacterial viruses is relatively a new science, but much information is being added to the field at an increasingly rapid rate. Frederick W. Twort is credited with discovering bacterial viruses, when he reported the observation of clear areas on Micrococcus culture plates (43,47). These clear areas were later found to be plaques caused by the lysis of the cells by the bacterial viruses (22).

d'Herelle (22) observed the clearing of normally turbid broth cultures of the bacteria which cause bacillary dysentery, and he concluded that the agent was "an invisible microbe, a filterable virus, a virus parasitic on bacterial cultures." He also showed that the viruses could not grow and reproduce on artificial media in the absence of a living system.

A method of assaying the titer of bacterial virus suspensions was devised by d'Herelle (22), which was based on the ability of the viruses to cause clear spots, or plaques, on an agar plate seeded with a "lawn" of susceptable bacteria. Although other assay techniques have been devised to count bacterial viruses, including electron microscopy (34), the plaque titration technique is still commonly used to enumerate infectious particles.

To estimate the accuracy of the plaque titration method, the efficiency of plating (EOP) of the virus must be considered.

Luria et al. (33), by using electron microscopy, showed that nearly every bacteriophage that can be seen under an electron microscope is capable of forming a plaque.

Probably the largest amount of information compiled about any one group of bacterial viruses is the data on the T-coliphages. The system consists of Escherichia coli strain B, and seven bacteriophages, T1-T7 (33). During the early study of bacteriophages, they were numbered type 1, type 2, and so on; however, these designations were abbreviated to capital T followed by the number. After the numbers reached 7, other designations became popular. It happened that the T-even phages seemed to be related, and the T-odd phages had certain properties in common.

STRUCTURE AND COMPOSITION OF THE T-COLIPHAGES

The suprastructure of the T-coliphages has been revealed by electron microscopy (7,33,53). The T-phages possess complex symmetry, a double strand of DNA, icosahedral heads, and a tail structure. Brenner et al. (9) further studied the structure of these phages and showed that the head of a T-even phage particle, in cross-section, is approximately hexagonal and capped by a hexagonal pyramid of protein. The head protein forms a semipermeable membrane enclosing the viral DNA. Also enclosed within the head protein or capsid, are several low-molecular-weight proteins and some amines. Ames, Dublin, and Rasenthal (1) found these amines to be spermidine

and putrescine.

The tail of the T-even phages is a complex structure composed of a rigid central shaft with a contractile tail sheath. A base plate is connected to the end of the tail, to which the tail fibers are attached (11).

Phages T1 and T5 have long, narrow, flexible tails which have no contractile mechanism, and the head of the virion is regularly icosahedral. Coliphages T3 and T7 have short tails and icosahedral heads (33).

ANTIGENICITY OF T-COLIPHAGES

The antigenic properties of the T-phages are revealed by certain serological reactions. Phage particles are antigenic, that is, when they are injected into the blood stream of certain animals they stimulate the production of antibodies which circulate in the blood stream and can react specifically with the antigen (26).

Kabat (26) illustrated that, according to cross-reaction experiments, the T-phages can be divided into four serological groups. Ordinarily, an immune serum reacts strongly with the homologous antigen. A cross-reaction may occur when an antibody reacts with another antigen that possesses a protein structure similar to the homologous antigen. Among the T-phages, the T-even strains, T2, T4, and T6 show cross-reactions one with the other. For example, Anti-T2 antiserum normally reacts with T2, but also reacts weakly with T4 and T6. T3 and T7 show cross-reaction; however, T1 and T5 show no cross-

reaction with any other T phages. Since the T-phages and host cells have no common proteins, no serological cross-reaction has ever been observed between bacterial viruses and bacterial host cells (24,43).

ADSORPTION

The process of adsorption of the bacteriophage to the host cell wall is a most important process and initiates the replicative cycle of the virus. Adsorption of phage particles is influenced by the physiological conditions of the bacteria and by the composition of the medium. In pure distilled water or at low concentrations of monovalent ions, most phages do not adsorb. The following optimum ionic concentrations were worked out by Tolmach (45,46) for the irreversible adsorption of some phages onto E. coli B.

T1	5×10^{-4} M	Mg^{++}, Ca^{++}, Ba^{++}, Mn^{++}, or Zn^{++}
	1×10^{-2} M	Li^{+}, Na^{+}, K^{+}, or NH_4^{+}
T2,T4,T6	1×10^{-1} M	Na^{+}, K^{+}, or NH_4^{+}
T3	1×10^{-3} M	Na^{+}, K^{+}, or NH_4^{+}
T7	1×10^{-2} M	Na^{+}, K^{+}, or NH_4^{+}; 1×10^{-3} M Mg^{++}

Because the phage particles have massive heads and small tails, the tails move more than the heads. Therefore, the tails are more likely to collide with the host first (37).

Anderson (2) demonstrated that bacteriophages T1 and T5 became attached to the host cells by the tip of the tail. However, prior to attachment, bacteria and phages must come

together and make an initial contact. The first of two possible mechanisms for the initial contact offered by Goodheart (20) involves the active participation of phages, cells, or both; whereas the second involves simple diffusion of the bacteria and viruses due to Brownian motion. The latter possibility was proven correct when Markham (36) showed that the phage does, in fact, contact the host as a result of random diffusion.

A relation was developed by Schlesinger (40) which stated that the irreversible binding of a phage at a given temperature is simply a function of the concentration of the phages and bacterial cells in the suspension.

The primary contact in the T-even phages involves the specific attachment of tail fibers to bacterial receptor sites, and the attachment of T3 and T7 is by the short tail structure (51). After the phage has become attached to the receptor site the virion remains for a period of time in a state of reversible attachment in which it can be cleaved from the bacterial wall with no loss of infectivity. After this period the virus particle is irreversibly adsorbed to the receptor site and if the virion is cleaved in this state, the infectivity of the particle is lost. The preliminary reversible attachment of T-phages is due to electrostatic forces which bind the phage particle to the cell while the process takes place that makes the bond permanent.

Considerable work has been performed to reveal the nature of the receptor sites on the bacterial cell walls.

Schlesinger (40) discovered that bacterial cell walls have specific receptor sites for phage attachment. He used mutant strains of bacteria which were isolated that either lost or gained the ability to adsorb phage particles. Also, removal of the cell wall with lysozyme prevented attachment of T-phages. Phage attachment to the protoplast was not possible because receptor sites in the cell wall were removed. The studies of Tolmach and Puck (46) showed that the adsorption reaction for most T-phages is pH dependent. Cellular carboxyl groups seem to be involved with T2 adsorption since the treatment of host cells with carboxyl blocking agents prevented attachment. Cells treated with specific amino-blocking reagents also lose their ability to bind T1 even though the affinity for T2 is unchanged. Thus host-virus specificity of some systems seems to be associated with the amino and carboxyl groups on the bacterial cell surface.

The probable chemical nature of the receptors for some of the T-phages was discovered by Weidel (51) while working with cell wall extracts of E. coli B. The receptors of T2 and T6 are probably lipoprotein, whereas those of T3, T4, and T7 are probably lipopolysaccharide. Isolated receptors of T5 apparently consist of bits of lipopolysaccharide covered by lipoprotein sheaths. The receptor site of T1 was not mentioned.

Weidel, Koch and Lohss (52) used 90% phenol to uncover the layer of E. coli B which exhibited viral receptivity. Chemical analyses revealed glucose, glucosamine, and another

unknown carbohydrate, probably a heptose, to be the components of the receptor material.

Receptor sites were studied by Bayer (4), who showed that in plasmolized E. coli cells, the protoplasmic membrane remained attached to the cell wall at 200 to 400 locations. This number range was approximately of the same magnitude as that for the number of receptor sites per cell for the T-phages. When bacteriophages T1 to T7 were added to such plasmolized cells, the virus particles adsorbed only to those areas at which the cell wall and protoplasmic membrane were attached. The chemical nature of these sites was not elucidated in that study.

Capsular slime and other layers external to the rigid wall are known to cover receptors and Bartell and Orr (3) used hydrolytic enzymes to remove those substances which may "mask" receptor sites.

PENETRATION

Once a viral particle adsorbs to its specific receptor site, it must then possess a mechanism of entry for the nucleic acid molecule. The penetration mechanism of the T-even viruses is well known; however, the mechanism of T-odd virus penetration is still unknown.

Hershey and Chase (23) demonstrated that only the penetration of the DNA of a phage is necessary to initiate a cycle of infection in a host. With the use of T2-phages containing S^{35}-labeled protein and P^{32}-labeled DNA, it was

shown that phage protein could be excised from infected cells devoid of DNA which had been injected into the cell. These experiments proved that only the viral DNA is necessary to initiate infection, and that the viral protein remains extracellular and apparently serves no other function once the DNA is injected into the cell.

The mechanism of DNA penetration first involves the dissolution of a portion of the bacterial cell wall. This is accomplished in T-phages by an enzyme carried in the tail. The enzyme has been called lysozyme (16) and does resemble egg-white lysozyme. After the cell wall has been dissolved, the actual DNA penetration is performed in the T-even phages by a sheath contraction in the tail mechanism which injects the viral DNA into the host cell. This is the only example of intrinsic motion found in bacterial viruses (13). This contraction, according to Kozloff et al. (30), can be brought about by extrinsic chemical stimuli such as peroxides, cadmium cyanide, and zinc complexes. During normal infection, the contractions are induced by the breaking of thiol ester bonds in the tail protein. This triggering of the tail sheath may be initiated by a small quantity of cellular material which possibly leaks from the opening in the cell wall made by the phage lysozyme. Wahl and Kozloff (48) discovered that the energy for the contraction of the tail mechanism is furnished by ATP which is contained in the tail sheath.

Stent (43) stated that the contractile tail sheath is a small "muscle," whose contraction resembles the interaction

between muscle proteins, actin and myosin. This statement was based on the fact that ethylene diaminetetra-acetate (EDTA) blocks both the contraction of actomyosin and the infection of bacteria by T-even phages. EDTA also prevents contraction of phage tail sheaths after adsorption of virus particles to isolated cell walls. However, the chemical composition and structure of phage tail protein do not in any way resemble that of actomyosin (43).

The injection process differs depending on the phage and Lanni (31) found that T5 only injects its DNA completely into bacteria that are synthesizing protein. If protein synthesis is inhibited, only a fragment of the phage DNA is injected, representing about 8% of the viral genome. This first injected fragment carries out some function which facilitates the entry of the remainder of the DNA, but the mechanism is still unclear, as is the penetration mechanisms for the other T-odd phages.

THE REPLICATIVE CYCLE

After the phage DNA penetrates the host cell, a series of events leads to the replication of the parent phage. In 1940, Delbruck (14) dissected the replicative cycle of the T-even phages by studying a mixture of phage particles and bacterial cells over a period of time. The concentration of the viruses remained constant for a time and then it suddenly increased to a new level and then again remained constant. The total cycle required about 45 min at 37 C.

Delbruck's one-step growth curve was divided by Doermann (15) into two periods; the latent period, and the time of rising titer. The latent period, which lasted 17 to 19 min, was subdivided into the eclipse and maturation stages (13,20).

The eclipse stage is the first post-infection stage in which the viral DNA begins to direct the manufacture of viral parts by the synthetic processes of the host cell. By using radioactive tracers, Koch and Hershey (27) found that protein synthesis for phage shell protein begins about 15 min after infection. Some early proteins are made prior to the first 15 min, but these are enzymatic in nature. Although protein synthesis of the cell proceeds without much delay after infection, DNA synthesis ceases during the first 7 to 8 min of the eclipse period and then resumes.

Hershey and Chase (23) found that in the eclipse stage, a pool of nucleic acid material is built up, which contains the direct precursor DNA of the completed phage. The pool receives its precursors of viral DNA from three sources; degraded host DNA, newly synthesized material from the medium, and DNA of the phage particle or particles that initiated the infection. With the use of radiophosphorus, Cohen (10) found that in cells infected with T2 or T4 the host cell DNA accounts for 14 to 18% of the progeny phage DNA, the medium contributes material for about 69 to 75% of the phage DNA, and the parental phage DNA adds a negligable amount of material to the progeny DNA.

During the eclipse stage of the T-even phages, which lasts approximately 20 min, the viral parts form and accumulate into pools. Time-lapse photography of an infected cell during the replicative cycle revealed, that the host cells begin to swell about midway through the latent period (6). This swelling is due to osmotic forces which cause a rapid uptake of water from the medium.

The parts begin their assembly into phage particles which marks the beginning of the maturation stage. Darnell and Luria (13) noted that one of the first steps in phage maturation is the condensation of the phage DNA from an extended form into a ball-like form. The internal head proteins respond to the DNA condensation, and in a process somewhat resembling crystallization, the phage protein forms the head around the DNA. The head assembly and the assembly of other protein parts takes place in localized, random, intracellular "factories."

The release of the mature phage progeny requires the lysis of the host cell, presumably due to an alteration of the permeability of the cell wall. An enzyme which may be similar to lysozyme is responsible and the synthesis of the enzyme is directed by the phage DNA. The parental phage need an enzyme to gain entry into the cell and the progeny phage also need the enzyme to escape from the host cell (15).

BIOCHEMISTRY OF THE LYTIC CYCLE

Once a bacterial cell is infected with a bacteriophage,

some of the host processes are turned off, others are accelerated, and others remain unaltered.

One of the processes that is changed is the synthesis of RNA since this change must occur before protein changes can occur. For a time after infection, cellular RNA synthesis remains quite active, but as the rate of phage RNA synthesis increases, the production of cellular RNA diminishes at a corresponding rate and there is no net RNA increase in the host cell (13,20).

Part of the soluble RNA of cells has been called messenger RNA (mRNA) by Jacob and Monod (25) and functions as the intermediate molecule that carries genetic information from the DNA to the ribosomes. The transcription of the viral DNA code into mRNA is catalyzed by a cellular DNA-dependent RNA-polymerase which synthesizes mRNA, some of which codes for early proteins, since mRNA synthesis precedes protein synthesis. Following mRNA synthesis, the information is translated into the specific amino acid sequence of a protein. Messenger RNA has a high rate of turnover, whereas, another soluble RNA that is part of the ribosome structure, ribosomal RNA (rRNA), is relatively stable. Brenner et al. (8) discovered that the new RNA synthesized in cells infected with T2 is viral mRNA.

Protein synthesis continues in infected cells even though ribosome synthesis ceases just after infection because of host DNA degradation. The protein that is synthesized is viral protein and is manufactured by the cell ribosomes

that exist at infection time. The ribosomes are non-specific and synthesize proteins according to the kind of mRNA present. The reason for differential protein synthesis of "early" and "late" proteins seems to involve the control of enzyme synthesis at the level of transcription of the genetic information from the base sequence of the viral DNA into mRNA, rather than at the level of translation of mRNA into the amino acid sequence of protein. However, the "late" genes are transcribed immediately after infection, as are the "early" genes, but the "late" mRNA becomes relatively inactive until later in the cycle (20).

The "early" proteins play a major role in completing the infective cycle, since they include the enzymes of a number of new metabolic pathways that appear in the infected cell, according to Stone and Burton (44). Since these enzymes do not exist prior to infection, the information for their synthesis apparently is contained in the phage DNA.

Examples of these new enzymatic pathways are, the degradation of host cell DNA by the action of deoxyribonucleases and the action of the enzyme deoxycytidylic deaminase which catalyzes the removal of the amino group from cytosine with the production of uracil. Some pre-existing metabolic pathways are more active as a result of the phage DNA. The "step-up" of a host enzyme system is illustrated by the increase in the production of DNA polymerase. In general, the function of the "early" enzymes seems to be the take-over

of the cell's metabolic processes and the direction of these metabolic processes to produce phage parts.

Although the above information is known concerning RNA synthesis, the mechanism by which phage DNA replicates in the host cell is not clear. Luria (32) proposed that the duplication of genetic material occurs and the product accumulates in pools, which is drawn upon when the maturation phase begins.

Watson and Crick (49), after proposing their double-helical model for the structure of DNA, suggested the semiconservative method of DNA replication in E. coli. According to this concept, the two strands disintwine and expose a nucleotide residue of each strand. Each exposed nucleotide, with the aid of DNA polymerase, binds complementary nucleotides which also become bound to the adjacent nucleotide. Two separate double strands eventually result. Later, Kosinski (28) observed, with the use of radiotracers, that phage DNA does, in fact, appear to replicate according to the Watson-Crick semiconservative model.

After "early" and "late" protein synthesis and viral DNA replication, the phage parts become assembled into the mature phage, the lysozyme lyses the cell, and the progeny emerges as mature infective particles.

STRUCTURE OF E. COLI CELL WALL

The multilayered ultrastructure of the E. coli B cell wall has been the subject of great inquiry and Bayer and Remsen (5), have further investigated it using the recently-developed freeze-etching technique. It was observed that the cell wall consists of lipoprotein, lipopolysaccharide, and peptidoglycan (mucopeptide) associated with a particulate protein. All of the amino acids in the protein component, with the exception of cystine, appear in E. coli cell wall extracts as well as diaminopimelic acid, hexosamine, and other unknown amino sugars (18,39,40). The lipid substances are found on the outer surface of the cell wall and the peptidoglycan faces the cytoplasmic membrane.

The surface of E. coli B appears smooth with minute pits, and Bayer (4) described the phenomenon as mosiacism. The "smooth" surface layer is the lipopolysaccharide-lipoprotein outer layer. The innermost layer of the cell wall contains the murein component of the wall. Protein is responsible for the beaded appearance of this layer and this protein contains lipid which binds the layer to the outer constituents of the cell wall. The outer membrane is lipoprotein in nature and closely resembles other biological membranes (38,41).

CHEMICAL AND BIOLOGICAL PROPERTIES OF DIMETHYLSULFOXIDE (DMSO)

Dimethylsulfoxide is a dipolar molecule that possesses a pyrimidal structure with sulfur, oxygen, and carbon atoms at the corners. It has a molecular weight of 73.13, melts

at 18.45 C, boils at 189 C, and has a density of 1.1014. DMSO is soluble in water, alcohol, chloroform, ether, and acetone (50). This aprotic solvent is usually obtained as a by-product of wood pulp manufacturing for paper, and is a useful solvent for acetylene, sulfur dioxide, and other gases. DMSO also dissolves many hydrocarbons and is useful in olefin preparations (37,42).

Biologically, DMSO has been shown to cross skin barriers in humans with little or no permanent tissue damage. However, primary irritation with redness, itching, and scaling sometimes does occur. Human volunteers have reported nausea, vomiting, cramps, chills, and drowsiness following skin absorption. Also, corneal opacities have been produced in experimental animals (42). Once in the epidermis, alterations in the protein structure can occur as a result of DMSO. These altered areas are flexible enough to pass this solvent in addition to substances mixed with it. Due to the size and spatial arrangement of the molecule, DMSO can permeate where bulkier solvents cannot (35).

In a study by Cummings, Chapman, and DeLong (11) DMSO was used to disrupt T-even bacteriophages. At a concentration of 67%, DMSO disrupted the phages into their component substructures with little loss of morphological integrity of the components.

In the field of cryobiology, DMSO has been shown to be a more useful agent than glycerol in protecting frozen material from freezing damage, due to the fact that it can

be incorporated into the water lattice of some proteins and other hydration sheaths (35).

In a very recent study by Feldman and Punch (17), E. coli cells were treated with varying concentrations of DMSO and Mg^{++}, and it was found that some misreading of mRNA occurred at optimum Mg^{++} concentrations. It was concluded, however, that DMSO and Mg^{++} act synergistically, and that DMSO alone may not cause any misreading at all at the ribosomal level.

The current literature, therefore, indicates that the major biological function of DMSO involves the factor of permeability, which is altered in such a way that this solvent passes readily across membranous barriers. However, the data concerning other biological functions of DMSO is still in a relative state of infancy and many questions remain unanswered.

MATERIALS AND METHODS

PREPARATION OF ESCHERICHIA COLI CULTURES

The Escherichia coli B ATCC 11303 culture used in this experiment was obtained from the Morehead State University stock culture collection. Inocula for the plaque assays were prepared daily in tryptose phosphate broth and incubated at 37 C for 18-24 hr.

PREPARATION OF T1-COLIPHAGE SUSPENSION

The bacteriophage used in this study was T1-coliphage ATCC 11303-B_1 obtained from the American Type Culture Collection. The phage suspensions were prepared by inoculating an 18-24 hr E. coli B broth culture with 1 ml of the stock phage suspension and then incubating at 37 C for 18-24 hr. After incubation, the suspension was centrifuged in an International centrifuge (Model HT) for 15 min at 10,000 rpm. The supernatant broth was then filtered through a Millipore filter and collected in a sterile test tube which was refrigerated until needed.

MEDIA

Bacto tryptose phosphate broth (Difco) was used for daily E. coli inocula and for phage production. Bacto tryptose agar (Difco) was used for the base layer of the plaque assay plates. Soft agar for the top layer of the plaque assay plates consisted of 0.7% Bacto agar (Difco).

PHAGE DILUTION SERIES

Each day that the plaque titration technique was used a phage dilution series of 7 test tubes (16 x 150 mm) containing 9 ml of tryptose phosphate broth was begun by adding 1 ml of the phage suspension to Tube 1 with a sterile 1 ml serological pipette. Tube 1 and all other mixtures were shaken with an electric mixer (Super Mixer - Fisher) and 1 ml from Tube 1 was transferred to Tube 2. The remainder of the dilution series was completed in a similar manner.

DETERMINATION OF VIRAL TITER

From each tube of the dilution series, 0.1 ml was transferred to test tubes (13 x 100 mm) containing 2.5 ml of soft agar and 0.5 ml of E. coli B culture. The soft agar tubes were previously melted and maintained at 45 C in a Thelco Model 28 water bath prior to the addition of E. coli B and T1-coliphage. After thorough mechanical mixing, the soft agar suspension was poured over a solidified base layer of tryptose agar in sterile petri plates (100 x 15 mm). The plates were incubated 24 hr at 37 C. The resultant plaques were counted in the plate containing between 30 and 300 plaques. The countable dilution for phage suspensions in this study was 10^{-6}.

CELL TREATMENT IN GROWTH MEDIUM

10% DMSO. The plaque titration was used as described above with the following modifications. Twenty soft agar

tubes were included in one experiment, 10 of which received no additional chemical and served as controls. To each of the remaining 10 tubes, 0.34 ml of 100% dimethylsulfoxide (DMSO) (Fisher Chem. Co.) was added, in addition to the bacteria and phage suspensions, and the resulting solutions in the tubes contained 10% DMSO. The 100% DMSO was added with a sterile disposable 1 ml tuberculin syringe. The subsequent procedures for plating, incubation, and counting were as described above.

Other DMSO concentrations. For the experiments in which other concentrations of DMSO were employed, the procedure described above was repeated except 0.03, 0.17, 0.51, and 0.76 ml of 100% DMSO were added for 1, 5, 15 and 20% DMSO concentrations, respectively.

Glycerol. The same procedure was repeated that was described for 10% DMSO, except the DMSO was replaced with 0.74 ml of 6M glycerol (Fisher Chem. Co.). The final mixture contained 1.28M glycerol.

Sucrose. The same procedure was repeated as described for 10% DMSO, except the DMSO was replaced with 1.48 ml of 3M sucrose (Fisher Chem. Co.). The final mixture contained 1.28M sucrose.

TREATMENT OF CELL SUSPENSION WITH DMSO

A 10 ml 18-24 hr *E. coli* B culture in tryptose phosphate broth was centrifuged and the supernatant broth was poured off. The pellet was resuspended in a 10 ml solution of 10% DMSO,

and the mixture was agitated for 15 min on a mechanical shaker. After agitation, the DMSO-E. coli B suspension was centrifuged, and after centrifugation, the DMSO supernatant was collected and filtered through a Millipore filter for subsequent biochemical examination. The pellet of cells was resuspended in 10 ml of physiological saline and the mixture was agitated and centrifuged as before. The saline wash was repeated and finally the pellet was resuspended in 10 ml of sterile tryptose phosphate broth.

The plaque titration method was conducted as described above using the DMSO-extracted cell suspension as the inoculum for the DMSO plates. No additional DMSO was added to the soft agar in this procedure. The control plates were prepared as usual with a normal 18-24 hr untreated E. coli B culture.

BIOCHEMICAL ANALYSES

The DMSO extract of the E. coli B cells, prepared as described above, was subjected to biochemical tests that detect the presence of carbohydrates, lipids, and proteins.

Carbohydrates. Four different qualitative tests were conducted in an attempt to reveal the presence of carbohydrates. For the Molisch test, 3 ml of the Molisch reagent was added to 3 ml of the DMSO filtrate in a 16 x 150 mm test tube. Concentrated H_2SO_4 was poured slowly down the inside of the tube. Glucose was tested as the control carbohydrate to test the reliability of the reagent.

For the Benedict test for reducing sugars, 5 ml of the

Benedict reagent was added to 5 ml of the DMSO filtrate in a 16 x 150 mm test tube. The tube was mixed and heated in a boiling water bath along with a tube containing the reagent and glucose as the control carbohydrate.

In the picric acid test, 1 ml of saturated picric acid and 0.5 ml of 1M Na_2CO_3 were added to 2 ml of the DMSO filtrate in a 16 x 150 mm test tube and heated in a boiling water bath for 10 min. Another tube containing glucose, picric acid, and Na_2CO_3 served as the carbohydrate control.

In the Seilwanoff test, 1 ml of the DMSO filtrate was heated in a boiling water bath along with 10 ml of the Seilwanoff reagent. Glucose in 10 ml of the reagent was also heated as the carbohydrate control.

Protein. The ninhydrin test was used for the qualitative detection of protein. On a piece of clean Whatman No. 1 filter paper, 5 drops of the DMSO extract were placed and then dried in a Lipshaw hot air oven. On another piece of filter paper 5 drops of 10% DMSO in distilled water were placed and dried in the same manner. Both pieces of filter paper were sprayed with 1% ninhydrin solution and heated for 5 min at 150 C.

Lipid. The qualitative test for lipids was carried out by using an ether extraction technique. In this test, 3 ml of the DMSO filtrate were placed in a 16 x 150 mm test tube along with 1 ml of diethyl ether. The tube was shaken and the ether layer was drawn off with a disposable 1 ml

sterile syringe and placed in a clean watchglass to evaporate.

One control tube contained one drop of tributyrin, 3 ml of distilled water, and 1 ml of diethyl ether. After the mixture was shaken, the ether layer was drawn off and placed in a clean watchglass to evaporate.

A second control tube contained 3 ml of 10% DMSO and 1 ml of diethyl ether. After shaking, the ether layer was drawn off and placed in a third clean watchglass to evaporate.

These same procedures were repeated on a DMSO extract from a larger volume of cells that were obtained from a 125 ml tryptose phosphate broth culture.

GROWTH CURVE

Four 16 x 150 mm test tubes and 4 matched Bausch and Lomb 1" cuvettes were filled with 9 and 25 ml of tryptose phosphate broth, respectively and autoclaved.

Two of the test tubes and 2 of the cuvettes were inoculated with a loopful of an 18-24 hr E. coli B broth culture, and the remaining tubes and cuvettes served as blanks. All were incubated at 37 C for 18 hr. The % transmittancy of the cultures was determined in a Bausch and Lomb Spectronic 20 spectrophotometer at a wavelength of 600 nm.

Figure 2 represents the growth curve of the E. coli B cells grown in the 16 x 150 mm test tubes since they were the kind of culture tubes used in the study and the growth curve was similar to that in the larger cuvette cultures.

STATISTICAL METHODS

The data collected were analyzed by means of the "t" test to compare the sample means. The significance levels of each group tested are found in their respective tables.

EXPERIMENTAL DATA

The results of extensive experimentation which were obtained in the initial phase of this study, show that the addition of 10% dimethylsulfoxide (DMSO) to the agar culture medium of the Escherichia coli B:T1-coliphage system caused more plaques to appear than appeared in controls. This effect was observed with E. coli inocula that had been incubated 18-24 hr at 37 C in tryptose phosphate broth with fresh T1-phage suspensions. Figure 1 shows a typical plate and plaques of T1-coliphage.

The experimental evidence for this effect is presented in Table 1, which shows that 100 plates containing 10% DMSO and 100 plates without DMSO were prepared and evaluated. Each experiment contained 10 test plates and 10 control plates. The phage was appropriately diluted to produce approximately 50 plaques per plate and the total plaques for each 10 plates is given in Table 1. The 10 experiments were conducted on five separate days with a separate E. coli culture for each test, but only two phage suspensions were used since the viral titer remains constant for months if kept refrigerated.

As a result of the treatment with 10% DMSO, 4802 plaques were obtained compared with 4336 without DMSO which is an increase of 10.8%.

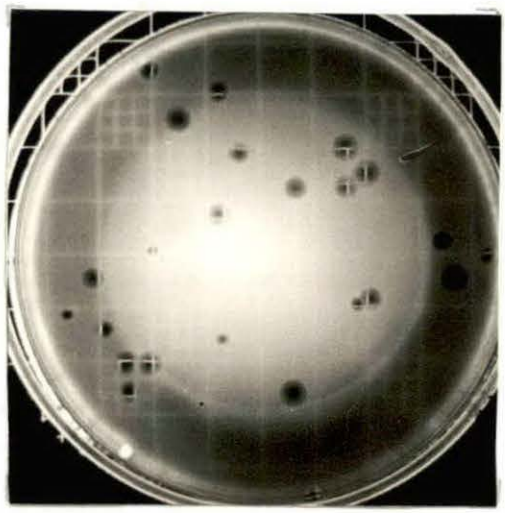

Figure 1. Typical plate and plaques of T1-coliphage.

Table 1. Effect of 10% DMSO on the number of T1-coliphage plaques.

Experiment Number[a]	Number of Plaques		Difference
	DMSO	Control	
1	516[b]	478	38
2	539	479	60
3	549	488	61
4	402	370	32
5	503	484	19
6	418	395	23
7	465	398	77
8	429	395	34
9	487	436	51
10	484	413	71
Total	4802	4336	466[c]

[a]Each experiment consisted of 10 test plates and 10 control plates.

[b]Total number of plaques in 10 plates.

[c]P=.05, df 18

Although 10% DMSO produced the above effect, higher and lower concentrations were less effective. Tables 2, 3, 4, and 5 contain results of the effect of 1%, 5%, 15%, and 20% DMSO, respectively.

Fewer plates were prepared than in the previous series but the procedure and cultures were similar. In the case of 1% DMSO (Table 2) 1534 plaques were counted on 30 plates, and the 30 control plates had 1533 plaques. The results of experiment 3 indicated a positive effect (11.3% increase) but the average included the negative results of experiments 1 and 2 which shows a difference between the DMSO treated plates and the controls of only 0.07%.

Table 2. Effect of 1% DMSO on the number of T1-coliphage plaques.[a]

Experiment Number	Number of Plaques		Difference
	DMSO	Control	
1	514	550	-36
2	541	559	-18
3	479	424	55
Total	1534	1533	1[b]

[a]Experimental conditions same as in Table 1.

[b]Does not approach a level of significance.

On 50 plates treated with 5% DMSO, 2368 plaques were recorded and compared to 2325 plaques on the 50 control plates. These values listed in Table 3 show an increase

of only 1.92%.

Table 3. Effect of 5% DMSO on the number of T1-coliphage plaques.[a]

Experiment Number	Number of Plaques		Difference
	DMSO	Control	
1	522	503	18
2	491	449	42
3	350	390	-40
4	557	448	109
5	448	550	-102
Total	2368	2325	27[b]

[a]Experimental conditions same as Table 1.

[b]Does not approach a level of significance.

Concentrations of DMSO greater than 10% produced variable results but they still produced fewer plaques than 10% DMSO.

In the case of 15% DMSO, 1124 plaques were observed on 30 test plates and the 30 control plates had 1091 plaques (Table 4). The percent increase for 15% DMSO is therefore 2.49%.

Table 4. Effect of 15% DMSO on the number of T1-coliphage plaques.[a]

Experiment Number	Number of Plaques		Difference
	DMSO	Control	
1	413	449	-36
2	508	468	40
3	203	174	29
Total	1124	1091	33[b]

[a]Experimental conditions same as Table 1.

[b]Does not approach a level of significance.

The highest concentration of DMSO that was tested in this study was 20%. Table 5 shows that at this concentation, 30 DMSO plates contained 752 plaques, whereas the 30 control plates possessed 818 plaques which gave a negative difference of 7.9%. At this concentration, the DMSO seems to be inhibitory to a certain degree since there were 66 more plaques on the control plates than on the test plates.

Table 5. Effect of 20% DMSO on the number of T1-coliphage plaques.[a]

Experiment Number	Number of Plaques		Difference
	DMSO	Control	
1	192	174	18
2	280	328	-48
3	280	316	-36
Total	752	818	-66[b]

[a]Experimental conditions same as Table 1.

[b]Does not approach a level of significance.

In the above experiments, the DMSO was added to the medium and may have exerted its effect throughout the entire viral replication process. An attempt was made to determine whether the DMSO effect required the continued presence of DMSO or simply a brief treatment of the bacterial cells prior to plating. In these experiments the E. coli B cells, which were treated with 10% DMSO and then washed before plating did not produce more plaques than a control of normal untreated E. coli B cells plated in the same manner (Table 6). In fact, fewer plaques were observed with the DMSO-treated cells rather than more plaques. On 30 experimental plates, 1652 plaques were counted, and on the 30 control plates 1708 plaques appeared for a minus difference of 3.39%.

Table 6. Plaque assay after E. coli B cells were treated with 10% DMSO and washed before plating.[a]

Experiment Number	Number of Plaques		Difference
	DMSO	Control	
1	535	593	-58
2	570	580	-10
3	547	535	12
Total	1652	1708	-56[b]

[a]Plaque assay conditions same as Table 1 except no DMSO in agar medium.

[b]Does not approach a level of significance.

Although the same effect of DMSO was not observed with

pretreated cells, the effect of DMSO in the culture medium was considered due to the extraction of some cell components. This possibility was investigated by extracting E. coli B cells with DMSO and subjecting the DMSO extract to biochemical analyses for carbohydrates, proteins, and lipids.

The Molisch test showed no indication of carbohydrates since there was no purple color upon addition of concentrated H_2SO_4. The purple color was very evident with the glucose control.

The Benedict test for reducing sugars was also negative since no yellow-orange color appeared as a result of the reduction of copper ions. The glucose control again gave a positive result.

Picric acid was also added to the DMSO filtrate to detect carbohydrates. No color change was observed upon heating, but the glucose control changed from yellow to a deep red color.

The final test for carbohydrates was the Seilwanoff test. As in the preceeding tests, the DMSO filtrate gave a negative result while the glucose control turned red when heated with the reagent.

The presence of protein in the DMSO extract was indicated by the ninhydrin test. Filter papers, containing both the DMSO extract and the casein control, had prominant purple spots.

An ether extraction technique was used for the

determination of lipid in the DMSO extract. A small amount of whitish residue was observed on a watchglass after evaporation of the ether layer that contained the DMSO extract. This residue was similar to the residue which remained after evaporation of an ether solution of tributyrin control. The ether solubility of this residue was considered as preliminary evidence for the presence of lipid in the DMSO extract. The same analytical procedures were repeated on a larger DMSO extract and the same results were obtained.

To determine whether the DMSO effect might involve increased osmotic pressure, equimolar concentrations of other organic substances were tested in the same systems. The results are presented in Table 7 and were variable for different substances. Sucrose produced fewer plaques than the control while glycerol caused an increase of 6.9%.

Table 7. Effect of other substances on the number of plaques.[a]

Experiment Number	Number of Plaques			
	Sucrose (1.28M)	Glycerol (1.28M)	DMSO (1.28M)	Control
1	410	436	473	431
2	401	440	448	417
3	350	471	476	406
Total	1161	1347	1397	1253
Difference	-92[b]	94[b]	144[c]	

[a]Experimental conditions same as Table 1.

[b]Does not approach a level of significance.

[c]P=.01, df 4

Some insight regarding the nature of the DMSO effect was expected from tests with a younger inoculum. These tests did, in fact, show that the culture age is a factor since a decrease in the number of plaques was observed with a 7 hr culture and 10% DMSO (Table 8). In the case of the 7 hr culture, the 30 DMSO plates possessed 1434 plaques, and the 30 control plates had 1476 plaques. The negative difference in the number of plaques between the experimental and control plates was 2.85%. However, with the 16 hr culture, the DMSO plates had 1600 plaques and the 30 control plates had 1409 plaques for a positive difference of 11.94%.

Table 8. Effect of culture age on the DMSO effect.[a]

Experiment Number	Number of Plaques			
	7 hr Culture		16 hr Culture	
	DMSO 10%	Control	DMSO 10%	Control
1	462	483	529	456
2	484	497	554	463
3	488	496	517	490
Total	1434	1476	1600	1409
Difference from Control	-42[b]		191[c]	

[a]Experimental conditions same as Table 1.
[b]Does not approach a level of significance.
[c]P=.01, df 4

Since the stage of growth reflects somewhat the nature

of the cells present, a growth curve of the E. coli culture was conducted to reveal the stages from which the 7 hr and 16 hr cultures were taken. Figure 2 is the growth curve obtained from the inoculum and shows that 7 hr cells were in the logrithmic phase and 16 hr cells were in the stationary phase.

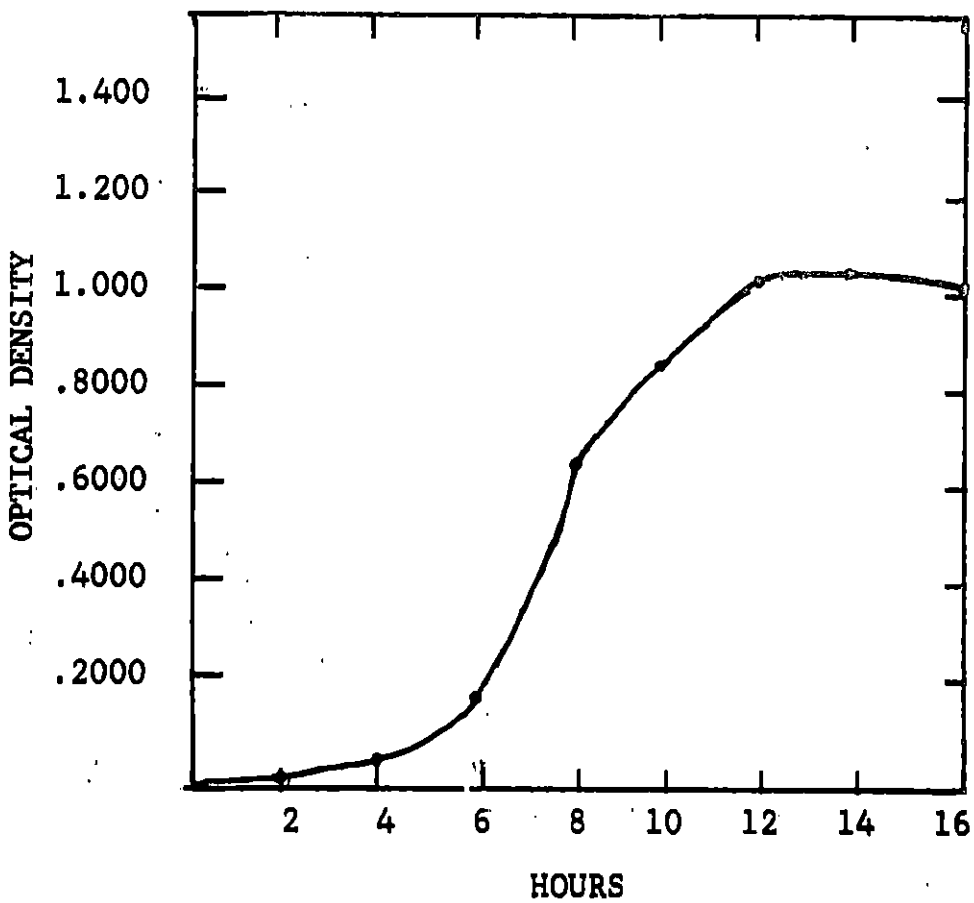

Figure 2. Growth curve of Escherichia coli B obtained from turbidity measurements.

DISCUSSION

The T1-coliphage:*Escherichia coli* B system is an extremely intricate system which remains relatively unexamined. However, with the use of the plaque titration technique, several significant facts have been determined as a result of this study. This technique as described by Cunningham (12), was expanded by including more tubes to fit this particular study and as a result the differences in the number of plaques could easily be observed. This difference in the number of plaques between the test plates and the control plates can be considered to be an increase in the sensitivity to T1-coliphage infectivity of a portion of the cell population.

The difference in the number of plaques was due to dimethylsulfoxide (DMSO) and the concentration of DMSO that proved most effective in increasing the phage infectivity was 10%. This study confirms this concentration as the most effective concentration as indicated in a preliminary study (Weldon and Reeder, unpublished data). Other concentrations of DMSO were much less effective in increasing infectivity as indicated by the insignificant difference in the number of plaques and by the negative differences. In several of the individual experiments, some of the differences seemed significant; however, the overall average of all the trials involved is the factor that was considered in this study.

A possible mechanism for the increased infectivity of the T1-coliphage might have as its basis the fact that DMSO dissolves protein and some lipid from the *E. coli* B cell wall.

These substances may mask some phage receptor sites on certain cells as described by Bartell and Orr (3). The removal of these masking substances by DMSO could therefore produce more cells with potential receptor areas, thus resulting in increased infectivity of the phage particles.

Rammler and Zaffaroni (37) indirectly described a possible mode of viral entry into animal cells by which the protein structure of the cytoplasmic membrane was altered by DMSO in such a way that allowed the solvent and certain substances mixed with it, including the whole virus, to pass through the membrane. Although the rigid cell wall of E. coli might present an additional barrier, T1-coliphage mixed with DMSO might somehow gain entry in this manner. Once inside, cellular proteases could act on the viral protein coat in such a way that would bring about the release of the viral DNA to initiate the replicative cycle of the virus.

The apparent requirement for incorporation of DMSO in the soft agar layer with the viral particles and the E. coli B cells may be a very significant factor for the DMSO effect. When the DMSO was incorporated into the soft agar tubes, which brought about a state of constant contact between the DMSO and the cells, an increase in infectivity was noted. On the other hand, when the DMSO was in limited contact with the E. coli B cells, i.e. the DMSO treatment and subsequent washing procedure, there was no apparent increase in infectivity of the T1-coliphage. In this case a cellular repair mechanism

may have operated in which the protein and possibly lipid portions of the cell wall that were dissolved by the dimethylsulfoxide were replaced by the cell before exposure to the phages.

Since the age of the E. coli B culture did have an effect on the action of DMSO, the physiological state of the cells may be a factor. A possible explanation on this basis is that in young actively-dividing cells that do not exhibit the DMSO effect, the cell wall is not completely formed and facilitates the active reproductive process (12). In this partially formed condition some of the phage receptors may not be formed, and the ones that have been formed have not been masked (3) by the addition of other cell wall materials as might be the case with older cells in the stationary phase of growth. Therefore only those cells that are sensitive to phage attack before DMSO addition are sensitive in the presence of DMSO and no increase in plaques is noted.

Another possibility is that the older cells present in the stationary phase of growth have decreasing permease activity (43) which limits the movement of nutrient material through the membrane from the medium. These old cells might not be capable of supporting phage replication even if attacked by the phage. Perhaps in these cells this decreasing permease activity could be replaced by an alteration of the cell membrane permeability by dimethylsulfoxide. Since 68% of the purines and pyrimidines that form the

progeny DNA are contributed by the media (10), this substitution of DMSO for permease may allow the "DNA material" and nutrients into the cell from the media. Such a process might allow the aging cells to support the process of viral replication. If a virus particle infected a dead cell, no progeny would be produced, and if the T1-coliphages attached to any nearly dead cells, they might have to be rejuvinated substantially to live throughout the period of viral reproduction. The function of DMSO might be associated with that process.

This study is an example of the potential use of dimethylsulfoxide as a tool that can be used in various fields. It may be the key that opens many doors in various fields such as medicine, microbiology, cryobiology, botany, and others yet to be discovered.

SUMMARY

The techniques employed in this study were designed in an effort to prove that 10% DMSO does indeed have an effect on the infectivity of the T1-coliphage as shown by the 10.8% increase in the number of plaques on the test plates. This significant increase in the number of plaques probably deals with alteration in the permeability of the E. coli B cell wall due to the constant presence of DMSO in the Escherichia coli:T1-coliphage system.

Other concentrations of DMSO were used in the Escherichia coli B:T1-coliphage system. However, at these concentrations no significant increase in plaques was observed.

The probability that DMSO alters the permeability of the cell wall is supported by the fact that protein and possibly some lipid were detected through biochemical analyses on DMSO extracts of E. coli B cells.

It was noted that the increased number of plaques was not due to a substantial increase in the osmotic pressure of the system, since sucrose and glycerol failed to produce the same effect.

The DMSO seemed to have no effect on the 7 hr inocula, whereas the 16 hr inocula did show a significant increase in plaques.

REFERENCES

1. Ames, B. N., D. J. Dublin, and S. M. Rasenthal. 1958. Presence of polyamines in certain bacterial viruses. Science. 127:814-818.

2. Anderson, T. F. 1953. The morphology and osmotic properties of bacteriophage systems. Cold Springs Harbor Symp. Quant. Biol. 18:197-203.

3. Bartell, P. F., and T. E. Orr. 1969. Distinct slime polysaccharide depolymerase of bacteriophage infected Pseudomonas aeruginosa. J. Virol. 4:580-584.

4. Bayer, M. E. 1968. Adsorption of bacteriophages to adhesions between wall and membrane of Escherichia coli. J. Virol. 2:346-356.

5. Bayer, M. E., and C. C. Remsen. 1969. Structure of Escherichia coli after freeze-etching. J. Bacteriol. 101:304-310.

6. Bayne-Jones, S., and L. A. Sandholzer. 1933. Changes in shape and size of Escherichia coli under the influence of a bacteriophage — A motion photomicrographic analysis of the mechanism of lysis. J. Exptl. Med. 57:279-304.

7. Bradley, D. E. 1967. Ultrastructure of bacteriophage and bacteriocins. Bacteriol. Rev. 31:231-308.

8. Brenner, S., F. Jacob, and M. Meselson. 1961. An unstable intermediate carrying information from genes to ribosomes for protein synthesis. Nature (London) 190:576-581.

9. Brenner, S., G. Streisinger, R. W. Horne, S. P. Champe, L. Barnett, S. Benzer, and M. W. Rees. 1959. Structural components of bacteriophages. J. Mol. Biol. 1:281-292.

10. Cohen, S. S. 1948. The synthesis of bacterial viruses: II. The origin of the phosphorus found in the deoxyribonucleic acid of the T2 and T4 bacteriophages. J. Biol. Chem. 174:295-303.

11. Cummings, D. J., V. A. Chapman, and S. S. DeLong. 1968. Disruption of T-even bacteriophages by dimethylsulfoxide. J. Virol. 2:610-620.

12. Cunningham, D. H. 1955. A laboratory guide in virology. Burgess Publ. Co. Minneapolis, Minn. 216p.

13. Darnell, J. E., and S. E. Luria. 1968. General Virology. John Wiley and Sons, Inc., New York, N. Y. 501p.

14. Delbruck, M. 1940. The growth of the bacteriophage and lysis of the host. J. Gen. Physiol. 23:643-660.

15. Doermann, A. H. 1948. Lysis and lysis inhibition with Escherichia coli bacteriophage. J. Bacteriol. 55:257-276.

16. Dunhill, P. 1967. Sequence of similarities between hen egg-white and T4 phage lysozymes. Nature (London) 215:621-622.

17. Feldman, W. E., and J. D. Punch. 1971. Effects of dimethylsulfoxide on poly U-directed polypeptide synthesis. Bacteriol. Proc. 1971:138.

18. Forsberg, C. W., J. W. Costerton, and R. A. MacLeod. 1970. Separation and localization of cell wall layer of a gram-negative bacterium. J. Bacteriol. 104:1338-1353.

19. Fowler, A. V., and I. Zaban. 1966. Effect of dimethylsulfoxide on the lactose operon in Escherichia coli. J. Bacteriol. 92:353-357.

20. Goodheart, C. R. 1969. An introduction to virology. W. B. Saunders Co. Philadelphia, Penn. 432p.

21. Green, M., M. Pina, and R. C. Kimes. 1967. Biochemical studies on adenovirus multiplication. J. Virol. 31:562-565.

22. Herelle, F. 'd. 1922. Discussion on the bacteriophage (bacteriolysin): I. The nature of the bacteriophage. Brit. Med. J. 2:289-297.

23. Hershey, A. D., and M. Chase. 1952. Independent functions of viral protein and nucleic acid in growth of bacteriophage. J. Gen. Physiol. 36:39-56.

24. Hirst, G. K. 1941. The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus. Science 94:22-23.

25. Jacob, F., and J. Monod. 1961. Genetic regulatory mechanism in the synthesis of protein. J. Mol. Biol. 3:318-356.

26. Kabat, E. 1961. Experimental immunochemistry. Thomas Publ. Co. Springfield, Ill. 286p.

27. Koch, G., and A. D. Hershey. 1959. Synthesis of phage precursor protein in bacteria infected with T2. J. Mol. Biol. 1:260-276.

28. Kozinski, A. W. 1961. Fragmentary transfer of P^{32}-labeled parental DNA to progeny phage. J. Virol. 13:124-134.

29. Kozloff, L. M., M. Lute, L. K. Crosby, R. Wong, and B. Stern. 1968. Critical arginine residue for maintaining the bacteriophage tail structure. J. Virol. 3:217-227.

30. Kozloff, L. M., M. Lute, and K. Henderson. 1957. Viral invasion: I. Rupture of thiol ester bonds in bacteriophage tail. J. Biol. Chem. 228:511-528.

31. Lanni, Y. T. 1965. DNA transfer from phage T5 to host cells: Dependence on intercurrent protein synthesis. Proc. Natl. Acad. Sci. U. S. 53:969-973.

32. Luria, S. E. 1951. The frequency distribution of spontaneous bacteriophage mutants as evidence for the exponential rate of phage reproduction. Cold Springs Harbor Symp. Quant. Biol. 16:463-470.

33. Luria, S. E., M. Delbruck, and T. F. Anderson. 1943. Electron microscope studies of bacterial viruses. J. Bacteriol. 46:57-77.

34. Luria, S. E., R. C. Williams, and R. C. Backus. 1951. Electron micrographic counts of bacteriophage particles. J. Bacteriol. 61:179-188.

35. MacGregor, W. S. 1967. The chemical and physical properties of DMSO. Ann. N. Y. Acad. Sci. 141:3-12.

36. Markham, R. 1962. Directiveness in biology on the molecular level. Amer. Sci. 50:570-575.

37. Rammler, D. H., and A. Zaffaroni. 1967. Biological implications of DMSO based on a review of its chemical properties. Ann. N. Y. Acad. Sci. 141:13-23.

38. Rogers, H. J. 1970. Bacterial growth and the cell envelope. Bacteriol. Rev. 34:194-214.

39. Salton, M. R. J. 1953. Studies of the bacterial cell wall: IV. The composition of the cell walls of some gram-positive and gram-negative bacteria. Biochim. Biophys. Acta. 10:512-523.

40. Schlesinger, M. 1932. Adsorption of bacteriophages to homologous bacteria. Z. Hyg. Immunit. 114:149-160.

41. Schnaitman, C. A. 1970. Protein composition of the cell wall and cytoplasmic membrane of Escherichia coli. J. Bacteriol. 104:890-901.

42. Stecher, P. G. 1968. The merck index, An encyclopedia of chemicals and drugs. Merck and Co. Inc. Rahway, N. J. 378p.

43. Stent, G. S. 1963. Molecular biology of bacterial viruses W. H. Freeman and Co. San Francisco, Calif. and London. 348p.

44. Stone, A. B. and K. Burton. 1962. Studies of the deoxyribonucleases of bacteriophage infected Escherichia coli. Biochem. J. 85:600-606.

45. Tolmach, L. J. 1957. Attachment and penetration of cells by viruses. Adv. Virus Res. 4:63-110.

46. Tolmach, L. J., and T. T. Puck. 1952. The mechanism of virus attachment to host cells. J. Amer. Chem. Soc. 74:5551-5557.

47. Twort, F. W. 1915. An investigation on the nature of the ultramicroscopic viruses. Lancet 189:1241-1247.

48. Wahl, R., and L. M. Kozloff. 1962. The nucleotide triphosphate content of various bacteriophages. J. Biol. Chem. 237:1953-1960.

49. Watson, J. D. and F. H. C. Crick. 1953. A structure for deoxyribose nucleic acid. Nature (London) 171:737-738.

50. Weast, R. C. 1968. Handbook of chemistry and physics. The Chemical Rubber Co. Cleveland, Ohio. 556p.

51. Weidel, W. 1958. Bacterial viruses with particular reference to adsorption and penetration. Ann. Rev. Microbiol. 12:27-48.

52. Weidel, W., G. Koch, and F. Lohss. 1954. Über die zellmembran von Escherichia coli B. Z. Naturforsch. 96:398-406.

53. Williams, R. C., and D. Frazer. 1953. Morphology of the seven T-bacteriophages. J. Bacteriol. 66:458-464.